Flow Cytometry Core
NIAMS, NIH

# Loading cells with Indo-1 for Ca flux with simultaneous extracellular antigen staining

## Uses and Rationale:

Calcium flux is used to measure activation of cells with a ligand/chemical. It measures immediate activation of the cell induced by the ligand/chemical, and then the cells returns to its "resting" state. This is visualized by flow cytometry as the ratio of bound indo-1 to free indo-1. Alternative methods for measuring this are using a 488 laser line with both Fura Red and Fluo 3.

## Materials:

Indo-1 AM
Cold Ca-containing PBS
Warm RPMI/10% FCS
Flow cytometer with UV laser line
Incubator/warm water bath

## Method:

1. Load cells with an Indo-1 at a concentration of 5ug/mL in 37 degree water bath for 45 minutes in media, preferably with a light resistant cover (otherwise, wrap tube in aluminum foil for incubation).
2. Spin down cells at 1500 RPM for 5 minutes
3. Wash 1x with cold PBS
4. Remove PBS, and leave 100uL in bottom of tube.
5. Vortex, add antibodies at normal concentrations you wish to stain for.
6. Incubate on ice in dark for 30 minutes.
7. Wash 1x with cold PBS.
8. Resuspend in approximately 500uL PBS…..do not fix cells.
9. Analyze on flow cytometer versus time, with ratio of blue indo to violet indo.

## Important technical notes:

Do PBS washings in calcium containing media
Use cold reagents after loading cells so that Indo-1 is not pumped out/fluxed inside the cell
Do not fix cells - you are doing a measurement on live cells, so fixing will negate this